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Published in:
Molecular Microbiology

DOI:
10.1111/j.1365-2958.2012.07984.x

Publication date:
2012

Molecular Microbiology (2012) 83(5), 884–893 ■
doi:10.1111/j.1365-2958.2012.07984.x
First published online 8 February 2012

MicroMeeting

A turning point for natural product discovery – ESF-EMBO research conference: synthetic biology of antibiotic production

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Summary

Synthetic Biology is in a critical phase of its development: it has finally reached the point where it can move from proof-of-principle studies to real-world applications. Secondary metabolite biosynthesis, especially the discovery and production of antibiotics, is a particularly relevant target area for such applications of synthetic biology. The first international conference to explore this subject was held in Spain in October 2011. In four sessions on General Synthetic Biology, Filamentous Fungal Systems, *Actinomyces* Systems, and Tools and Host Structures, scientists presented the most recent technological and scientific advances, and a final-day Forward Look Plenary Discussion identified future trends in the field.

Introduction

Synthetic biology is considered as the major future trend for biotechnology: our newly increased ability to sequence and (most importantly) synthesize entire genomes enables a new engineering-style approach to manipulating biological systems (Leonard *et al.*, 2008; Khalil and Collins, 2010; Tyo *et al.*, 2010; Tsvetanova *et al.*, 2011). At the moment, much of the potential of Synthetic Biology is realized only at the level of proof-of-principle studies and general plans, but some areas of microbiology are already geared up for applied synthetic biology. The field of secondary metabolism, especially the discovery and production of bioactive compounds, including antibiotics, is particularly well positioned for such a strategy. Biosynthetic pathways for secondary metabolites are modular at multiple levels, and therefore are a natural target for re-engineering and the synthetic creation of additional chemical diversity (Carothers *et al.*, 2009; Fischbach and Voigt, 2010; Gao *et al.*, 2010; Dhamankar and Prather, 2011; Medema *et al.*, 2011a,b; 2012).

On 2–7 October 2011, more than 100 synthetic biologists and fungal/actinomycete natural products biologists met at the Hotel Eden Roc, Sant Feliu de Guíxols (Costa Brava), Spain, to explore the state of the art and future directions of the field in a meeting co-hosted by the European Science Foundation (ESF) and the European Molecular Biology Organization (EMBO). A perfect venue overlooking the rocky cliffs of the Mediterranean Sea and the beautiful late-summer weather provided the ideal environment to bring together these two very different communities to exchange ideas and to form new collaborations. The aim was more than achieved, with talks and posters from established group leaders, as well as the young scientists who will lead the future of Synthetic Biology. Many talks included unpublished and hot-off-the-bench data and inspired all participants to lively discussions. Forty-seven posters were presented, and each poster author gave a 1 min talk on their work (without slides). This was a widely approved highlight of the whole conference, which led to many enthusiastic interactions during the subsequent poster sessions.

Three prizes were awarded to young researchers: the SGM young speaker prize was awarded to Daniel Scharf, PhD student, Leibniz Institute for Natural Product

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Research and Infection Biology e.V., Jena, Germany, for his talk entitled: Gliotoxin pathway reconstruction as a prerequisite for pathway engineering. The *Nature Reviews Microbiology* poster prize was awarded by Andrew Jermy (NRM senior editor) to Eva-Maria Niehaus, PhD student, Westfälische Wilhelms-Universität Münster, Germany, for her poster with the title: Molecular and chemical characterization of secondary metabolite gene clusters in *Fusarium fujikuro*: the fusarin gene cluster. The *EMBO Reports* poster prize was awarded to Tina Strobel, PhD student, Albert-Ludwigs-University, Freiburg im Breisgau, Germany, for her poster with the title: Identification of a highly flexible glycosyltransferase from *Saccharothrix espanaensis*.

Another important feature of the conference was the active participation from industry. Eighteen industrial researchers attended the conference, with six presenting short talks or posters, reflecting a (re-)growing interest in natural products in the light of the recent advances of synthetic biology approaches in the field.

The meeting was an excellent illustration of how far we have proceeded in the last couple of years, both in our understanding of secondary metabolite biosynthesis and in our tools for large-scale genetic manipulation. At the same time as bringing together synthetic biology and natural products, and inspiring collaborations within the natural products community, it also identified important bottlenecks in the current synthetic biology pipeline and important limitations of our biological understanding of the relevant biosynthetic pathways and their regulation, which will benefit from the joint attention of both groups of experts.

Meeting lectures

General Synthetic Biology

The conference was opened by **Roy Kishony** (Harvard University, USA) with his inspiring presentation of new data on 'The ecology of antibiotics', using experimental and computational modelling strategies to explain the natural evolution of antibiotics resistance, answering the intriguing question, why haven't all bacteria become resistant yet? The complex spatial and temporal dynamics of antibiotics gradients lead to significant growth advantages of antibiotic-*sensitive* strains even in the presence of antibiotics producers. Roy also presented his very recent work on *Streptomyces* inter-species interactions (Vetsigian *et al.*, 2011). Sixty-four *Streptomyces* strains were isolated from soil and observed for sender–receiver interactions using antibiotic production, resistance and growth. The results show that the interactions evolve quickly, and mathematical modelling of the ecological dynamics of antibiotic production and resistance suggested that in a diverse *Streptomyces* community the interaction network is evolutionarily dynamic. Therefore the antibiotics produced by *Streptomyces* are forever diverse. This was good news to those of the audience looking for novel antibiotics!

Christina Smolke (Stanford, USA) presented a large collection of tools for the control of gene expression in yeast, mostly based on RNA and its secondary structure, and showed how to use these switches to control antibiotic production. She illustrated this, for example, by RNA-based control modules to control translation using the RNase Rnt1p, which cleaves the conserved sequence in an RNA hairpin (Fig. 1) (Babiskin and Smolke, 2011a). Based on these data Christina argued that the flexibility of RNA molecules as sensors, computing devices and actuators, and their compatibility with the existing endogenous regulatory machinery, makes them particularly promising as portable programmable tools for the design and engineering of tightly controlled metabolic pathways.

Markus Schmidt (Biofaction and IDC, Austria) discussed 'Biosafety and Public Dialogue in Synthetic Biology', raising awareness of an important but often neglected issue by showing examples of public engagement through animation, films and art. Although synthetic biology of antibiotics itself is usually not perceived as a threat to society, this is still an important issue, given the generally cautious attitude of the public towards large-scale genetic engineering. To illustrate the pro-active approach taken by researchers in the field, Markus showed an animation created by the consortium SYNMOD in EuroSYNBIO (ESF funded) to raise awareness concerning the importance of our search for novel antibiotics that can combat antibiotic-resistant pathogens. At the Bio:fiction Science Art and Film Festival held in Vienna in May 2011, over 52 short films related to synthetic biology were shown ranging from documentary films to animations and pure science fiction (http://www.bio-fiction.com). Artists also presented 10 pieces of art related to synthetic biology at the art-science exhibition 'synth-ethic', produced by Markus and curated by Jens Hauser, in the Museum of Natural History in Vienna, May to June 2011, demonstrating an extremely diverse interpretation of synthetic biology by international artists (http://www.biofaction.com/synth-ethic/).

Arnold Driessen (University of Groningen, The Netherlands) reported data on the engineering of *Penicillium chrysogenum* for the overproduction of penicillin. There are about 830 transporters encoded in the genome of *P. chrysogenum*, including 49 ABC transporters. From these ABC transporters, 23 were selected based on their expression profile in penicillin-producing conditions and deleted. One showed a lowered production of penicillin and may represent a potential bottleneck transport reactions during penicillin biosynthesis. It is now the target of overexpression studies (Weber *et al.*, 2011).

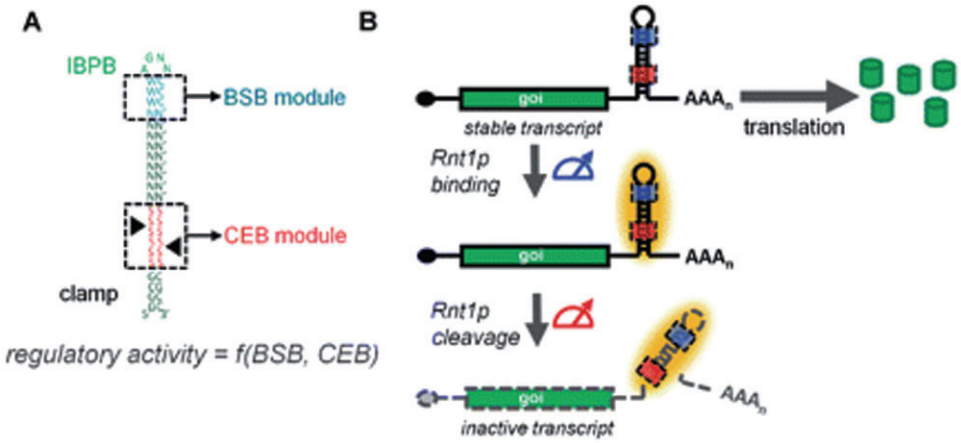

Fig. 1. Mechanism of the Rnt1p-based post-transcriptional genetic control elements.
A. Consensus structure of the Rnt1p substrate hairpin, indicating the two tuneable modules CEB (red) and BSB (blue), and the initial binding and positioning box (IBPB) (green). The cleavage sites are indicated by black triangles.
B. Example of a regulatory element implemented using the Rnt1p substrate hairpin in the 30-UTR of a gene of interest (goi) to reduce protein levels through transcript destabilization by endonucleolytic cleavage. Mutations in the BSB (blue box) and CEB (red box) modules are expected to tune the hairpin's affinity for Rnt1p RNase (blue dial) and its sensitivity to cleavage (red dial) respectively.
Figure modified from Babiskin and Smolke (2011b).

Jörg Stelling (ETH, Switzerland) provided a systems biologist's perspective on synthetic biology, discussing to which extent can we come up with a biological design that really works, in the face of lack of knowledge, system sloppiness, stochasticity and limited insulation, by exploiting general design principles. Jörg argued that to provide synthetic biology with the reliable, predictable behaviour that characterized classical engineering, it will be necessary to complement the molecular standard parts ['biobricks' (Shetty *et al*., 2008)] with similarly standardized mathematical models. The potential of a quantitative engineering approach was evidenced by the development of computational tools to automatically and rationally design a genetic circuit with predictable regulatory properties (Fig. 2) (Marchisio and Stelling, 2011).

Several short talks in this session presented new assembly methods for large DNA fragments. **Nili Ostrov** from Virginia Cornish's laboratory (Columbia University, USA) gave an overview of the reiterative recombination technology for assembly of an in principle unlimited number of DNA fragments into the chromosome of *Saccharomyces cerevisiae* (Wingler and Cornish, 2011), which enables construction of multi-gene biosynthetic pathways and large combinatorial libraries in a user-friendly manner and using a minimal number of selective markers. **Todd Peterson** (Life Technologies, USA) described the *in vivo* large-scale assembly of DNA sequences leveraging the TAR system in yeast, as well as a low-order, enzymatic *in vitro* assembly for vector construction. This topic was further elaborated by **Ralf Wagner** (GeneArt/Life Technologies, Germany), using the example of synthetic lantibiotics. **Barry Canton** (Ginkgo Bioworks, USA) presented the logistic and computational challenges of establishing a pipeline for organism engineering in an industrial context, with the ambitious aim of automating the steps from initial design to the final engineered microorganism as far as possible. Another inspiring talk was from **Karsten Temme** from Chris Voigt's laboratory (UCSF/MIT, USA) who discussed the re-engineering of a complex biological system, the nitrogen fixation cluster of *Klebsiella oxytoca*, by replacing the entire natural regulatory machinery of this very large and fragile gene cluster by synthetic elements.

Filamentous Fungal Systems

The second day illuminated a wide range of fungal model systems for secondary metabolite production. **Nancy Keller** (University of Wisconsin, USA) used case studies from a range of different *Aspergillus* species to illustrate the power of developmental biology in unlocking the treasure chest of fungal secondary metabolites, especially using regulatory genes/elements characterized by her group. One such method was to mutate proteins involved in chromatin modification. By mutation of *cclA*, a gene involved in a histone-3-lysine-4-methylation, nine secondary metabolites were found to be produced (Bok *et al*., 2009), and one of them, an emodin derivative, showed antifungal activity against human pathogenic fungi (Giles *et al*., 2011). This method shows the promises of targeted engineering for awakening the biosynthetic potential of the huge collection of secondary metabolite biosynthesis gene clusters identified by genome sequencing.

Axel Brakhage (Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institut,

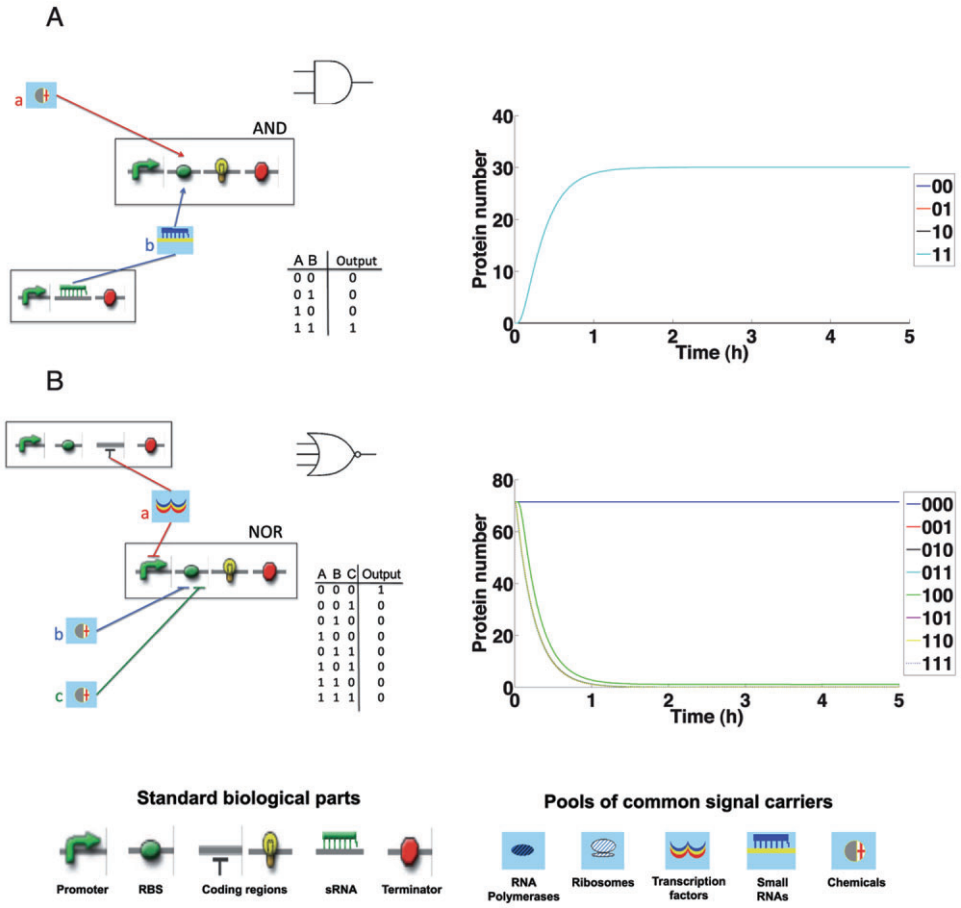

Fig. 2. Schematic representation of the creation of biological Boolean gates using standardized biological parts. Panels (A) and (B) illustrate the possible composition of an AND gate (two inputs; A) and a NOR gate (three inputs; B), as well as the corresponding read-out in terms of the expected protein levels in different input conditions. Inputs can be of different forms, including transcription factors, small RNAs and chemicals. (Figure modified from Marchisio and Stelling, 2011.)

Germany) demonstrated another important strategy for the activation of silent gene clusters, using the overexpression of pathway-specific regulatory factors. One of his examples was the overexpression of a regulator, ScpR, which awakened the production of the polyketide asperfuranone although the gene was localized in an NRPS-encoding gene cluster (cross-pathway regulator) (Bergmann *et al*., 2010). Another strategy was to change the cultivation conditions to mimic those of the host for the pathogenic fungus *Aspergillus fumigatus*, e.g. by oxygen limitation (Vödisch *et al*., 2011). Using proteome analysis, 117 proteins with altered protein abundance in hypoxic conditions were identified, including the genes which are involved in the biosynthesis of pseurotin A, whose biosynthesis genes are silent under normal growth conditions. A third example of awakening sleeping gene clusters exploited the co-cultivation of microbes (*Streptomyces*) and *Aspergillus*, which induced the production of orsellinic acid and its derivatives that are usually not produced in wild-type fungi. The co-cultured *Streptomyces* alters the histone modification of the fungus and thus triggers/enhances secondary metabolite production (Nützmann *et al*., 2011).

Gerhard Braus (University of Göttingen, Germany) illustrated the complex regulatory elements in *Aspergillus nidulans*, and how these regulators are controlled by different environmental signals and also can effect each other. In particular, he discussed the often very intricate interplay between developmental regulators and secondary metabolite production, as represented by the VelB–VeA–LaeA protein complex that co-ordinates sexual fruiting body formation and secondary metabolite production (Fig. 3) (Sarikaya Bayram *et al*., 2010; Bayram and Braus, 2012). As another example of a pleiotropic regulator affecting secondary metabolite production and development, Gerhard introduced the COP9 signalosome (Braus *et al*., 2010). Combining data from transcription, protein and metabolite analysis, the fungal COP9 signalosome was shown to be required for protection from oxidative stress and hormone regulation early in development and in later

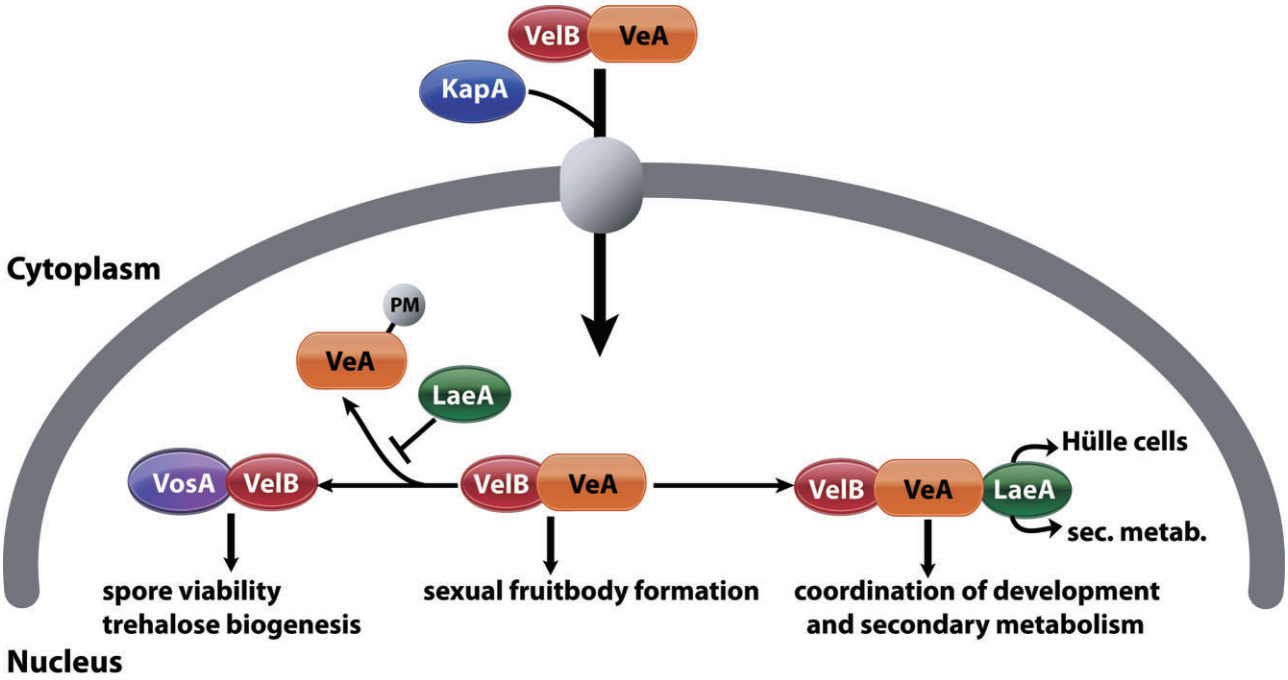

Fig. 3. Cartoon of the light-regulated circuitry based on interactions of VelB, VeA and LaeA proteins that integrates morphological differentiation and secondary metabolism in *Aspergillus nidulans*. (Figure from Sarikaya Bayram *et al.*, 2010.)

development, for the control of secondary metabolite production and cell wall arrangement (Nahlik *et al.*, 2010).

In a short presentation in this session, **Daniel Scharf** from Axel Brakhage's laboratory (Leibniz Institute for Natural Product Research and Infection Biology e.V., Hans-Knöll-Institut, Germany) gave his award-winning talk on the biochemical characterization of the newly characterized enzymes involved in the biosynthesis of the bioactive disulphide motif in gliotoxin, a virulence factor from *A. fumigatus* (Scharf *et al.*, 2010; 2011). **Stefan Olsson** (University of Copenhagen, Denmark) shared case studies of discovering new bioactive compounds from fungi by exploring extreme environments (soil from Greenland potato fields) and unusual niches (the hyphosphere of Vietnamese rice plants), and emphasized the importance of inter-species interactions in the activation of secondary metabolite production. **Vera Meyer** (Berlin University of Technology, Germany) stressed that resistance development needs to be considered at an early stage of drug development and discussed how differences in survival strategies determine which target organisms will be sensitive or resistant against antibiotics (Ouedraogo *et al.*, 2011).

Actinomyces *Systems*

The third day of the meeting was dedicated to exploring the diversity of actinomycete secondary metabolism. The scene was, however, set by two talks that focused on non-actinomycete bacteria with particularly diverse secondary metabolomes. **Jörn Piel** (University of Bonn, Germany), who presented the astonishing chemical richness of secondary metabolites produced by animal-associated bacteria, mostly symbionts of marine sponges (Gurgui and Piel, 2010; Piel, 2011). Metagenomics revealed pathways for compounds, including psymberin and the misakinolides, encoded by symbiont gene clusters. Single-cell analysis consisting of cell separation by flow cytometry, single-cell genome amplification and PCR-based localization of biosynthetic and 16S rRNA genes was shown to be a useful method to associate secondary metabolites with individual producers in complex consortia of uncultivated bacteria.

Rolf Müller (Helmholtz Institute Pharmaceutical Research Saarland, Germany) gave a broad-ranging overview of the engineering of biosynthetic pathways mostly in myxobacteria (Weissmann and Müller, 2010), and also illustrated the power of improved bioanalytical strategies for identifying novel secondary metabolites by comparative metabolomics. For example, the identification of products for previously believed cryptic secondary metabolite pathways was demonstrated in *Myxococcus xanthus*, by a sophisticated secondary metabolome mining approach involving comparative high-resolution mass spectrometric analysis of wild type and PKS/NRPS mutants of the target strain (Cortina *et al.*, 2012). Novel structures were also obtained by mutasynthesis. Starting from the elucidated biosynthetic pathway of cinnabaramides, a class of protea-

some inhibitors produced by terrestrial streptomycetes, one enzyme, CinF, which acts in the reductive carboxylation of octenoyl-CoA and producing 2-carboxyoctanoyl-CoA was mutated and chemically synthesized fatty acid analogues were used to complement the mutant. Grown in the presence of (E)-6-chlorooct-2-enoic acid (E)-8-chlorooct-2-enoic acid, and their corresponding *N*-acetylcysteamine (NAC) thioesters, new chlorine-containing compounds were produced, which showed better inhibitory activity towards all three proteolytic subunits of the proteasome (Rachid *et al*., 2011).

Returning to the evolutionary theme of the first day, **Peter Leadlay** (Cambridge University, UK) emphasized that successful natural products have been shaped and preserved by natural selection, but that the natural repertoire represents only a limited sampling of the entire available chemical space. He then illustrated how synthetic biology approaches, in particular the modular engineering of polyketide synthases, can be used to extend the range of accessible chemical diversity. For example, intermediates of the natural biosynthetic pathway could be accessed in large amounts from a polyketide synthase, 6-deoxyerythronolide B synthase 3, using non-hydrolysable pantetheine and *N*-acetyl cysteamine mimics of the natural (methyl) malonyl extender units recruited for polyketide formation as competitive substrates (Tosin *et al*., 2010). The current state of our biochemical understanding of complex biosynthetic pathways was demonstrated by the successful *in vitro* reconstitution of the synthesis of a tetronate polyketide, 3-hexadecanoyl-5-hydroxymethyltetronic acid (Sun *et al*., 2010). *In vitro* synthesis of such a polyketide antibiotic opens the door to possible recombination/redesigning of chemical structures.

Wolfgang Wohlleben (University of Tübingen, Germany) presented unpublished data on redirecting flux towards balhimycin production, especially by modifications in primary metabolism, which resulted in overexpression of biosynthesis pathway enzymes (Thykaer *et al*., 2010). Another antibiotic, kirromycin, was also studied for its unusual trans-AT enzyme, KirCII, which regiospecifically incorporates the unusual extender unit ethylmalonyl-CoA (Musiol *et al*., 2011). These enzymes will be useful for building diverse module libraries of PKSs.

Diverse synthetic biology approaches were also presented in the short talks. **Anna Eliasson Lantz** (Technical University of Denmark, Denmark) spoke of her recent work on the construction of a synthetic promoter library by randomizing the −10 and −35 and the 17 bp spacer region of the promoter. A library of promoters was inserted in front of a promoterless activator for actinorhodin biosynthesis, *act*II-ORF4, and visually screened for actinorhodin production. Interestingly, half of the resulting isolates did not grow, but from those that did, a collection of promoters with a wide range of defined strength was obtained. **Justin Nodwell** (McMaster University, Canada) presented unpublished data on his small-molecule interrogation of the life cycle of *Streptomyces coelicolor*. Surprisingly, weak inhibitors of fatty acid synthesis awakened the production of several cryptic antibiotics and affected the expression of pleiotropic regulatory genes. **Hiroyasu Onaka** (Toyama Prefectural University, Japan) presented current work on the high-throughput engineering of NRPS using goadsporin as a proof of concept (Onaka *et al*., 2005; Onaka, 2009). Fifty-two analogues of goadsporin were engineered by deletion and mutation of the biosynthetic pathway genes. Further investigation and redesigning is underway to find active novel compounds.

Tools and Host Structures

On the final day, **Luis Serrano** (CRG, Spain) started the session by a systems-biology overview of our current ability to create a comprehensive quantitative picture of a microbial systems (specifically *Mycoplasma pneumoniae*), including the quantification of protein and transcript levels, identification of small RNAs but also of translation rates, protein half-lives and protein–protein and protein–DNA interactions, using a combination of technologies, ranging from next-generation sequencing to electron microscopy tomography (Fig. 4) (Güell *et al*., 2011; Maier *et al*., 2011). From this analysis, predominantly post-transcriptional regulation, rather than post-translational mechanisms, was found to control cellular mRNA-to-protein abundance ratios, and unusual subunit stoichiometries indicate protein complex dynamics and suggested possible moonlighting for several ribosomal proteins.

Haruo Ikeda (Kitasato University, Japan) illustrated how large-scale engineering can improve even established hosts for secondary metabolite production, showing how mega-deletion mutants of the chromosomal ends can achieve improved industrial potential (Komatsu *et al*., 2010). Using this superhost, an epi-isozizaene synthase (SAV 3032) was reintroduced under the promoter rpsJp (sav4925); while this synthase is normally silent, under these conditions it produced the previously characterized oxidized epi-isozizaene metabolites (4R)- and (4S)-albaflavenols and albaflavenone, as well as a novel doubly oxidized epi-isozizaene derivative, 4β,5β-epoxy-2-epi-zizaan-6β-ol, which is most likely formed by oxidation of (4S)-albaflavenol (Takamatsu *et al*., 2011).

Kristala Jones Prather (MIT, USA) demonstrated the versatility of new protein devices for biosynthetic pathway design in an example of increasing the productivity for a specific small molecule of industrial interest in a heterologous host, using glucaric acid as an example. A recombinant pathway was engineered to produce the target

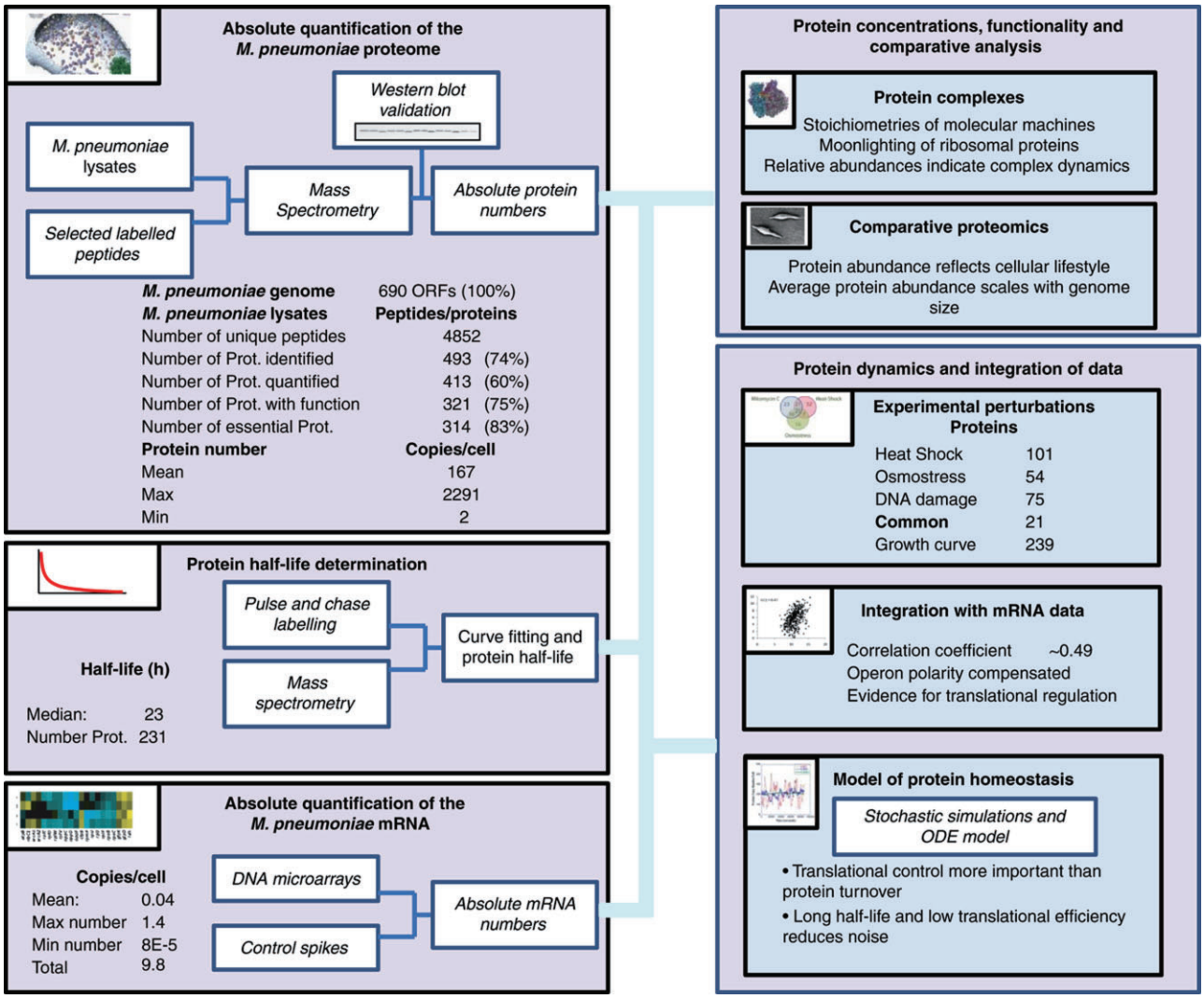

Fig. 4. Overview of the large-scale quantitative assessment of mRNA and protein abundance and dynamics in *Mycoplasma pneumoniae*, including a summary of the major results. (Figure from Maier *et al.*, 2011.)

compound in *Escherichia coli* by placing the required enzymes from diverse sources onto a synthetic scaffold held together via eukaryotic protein–protein interaction domains (Dueber *et al.*, 2009). The flux from glucose to *myo*-inositol was redirected towards glucuronic acid by introducing *myo*-inositol-1-phosphate synthase from *S. cerevisiae* and a mouse *myo*-inositol oxygenase, and subsequent introduction of uronate dehydrogenase from *Pseudomonas syringae* or *Agrobacterium tumefaciens* str. C58 lead to glucaric acid (Moon *et al.*, 2009a,b; 2010).

Michael Fischbach (UCSF, USA) presented a wide-ranging perspective on the discovery and characterization of secondary metabolites based on genome sequences (Fischbach and Voigt, 2010) emphasizing, the broad (and largely unexplored) phylogenetic distribution of secondary metabolite biosynthetic gene clusters, many of which encode for biosynthetic machineries that are larger than a ribosome and must have immense evolutionary benefits for their carrier organisms if they show such persistent evolutionary conservation. Presenting an unpublished analysis of the global distribution of secondary metabolite gene clusters in more than 1000 completely sequenced genomes, he identified underexploited clades with unexpectedly rich biosynthetic capacities, and provided new insights into the pervasive shuffling and recombination of the biosynthetic machinery across the bacterial kingdom, which will serve as an important guide in synthetic biologists' attempts to further improve on the natural diversity of compounds.

Beatrix Suess (Frankfurt University, Germany) presented the engineering of riboswitches which respond to small molecules, in particular tetracycline and neomycin. Many aptamers are known to bind to small molecules but not all have the function to be used as riboswitch. The

mechanism by which the aptamers can become a riboswitch was described by using aptamers that respond to neomycin (Weigand *et al*., 2011). In addition to high binding affinity for the ligand (preferably in the nanomolar range), it is also necessary that the ligand binding induces a major conformational change, and has the ability to control the degradation of mRNAs and rRNAs. To engineer such switches, one tetracycline aptamer was linked to a hammerhead ribozyme with variable linker sequences. The resulting hybrid was mutated and selected *in vitro* for 11 rounds to identify variants that gave tetracycline-dependent cleavage. Different linker sequences were determined that were cleaved at 1 μM tetracycline and were functional in yeast as demonstrated using a GFP reporter system (Wittmann and Suess, 2011).

Chris Voigt (MIT, USA) challenged the natural product community to be more daring and ambitious in their adoption of synthetic biology concepts and technologies, illustrating the potential of large-scale engineering of biology by several examples of unpublished data from his group that had succeeded in completely refactoring complex biological systems, ranging from nitrogen fixation (which was also presented in more detail by **Karsten Temme** on the first day) and light harvesting, to protein secretion systems. He presented the necessary computer-aided design (CAD) tools, including algorithms to optimize codon usage and ribosomal binding sites, and versatile promoter and terminator prediction tools (Clancy and Voigt, 2010). He also presented experimental components for such an enterprise, for example light-controlled systems using the phytochrome signalling network of *Arabidopsis thaliana* (Tabor *et al*., 2011), a genetic circuit using quorum sensing (Tamsir *et al*., 2011), and a large library of T7 promoters of different well-characterized promoter strength. However, he also highlighted the various pitfalls along the way, stressing that natural products like antibiotics are of 'nice intermediate complexity' to allow a successful application of synthetic biology, being produced by pathways that are more complex than most targets of present-day metabolic engineering, but less daunting than whole-genome design projects.

In the short talks, **Marnix Medema** from the Takano group (University of Groningen, The Netherlands) presented some of the bioinformatics tools that underlie the genome-based discovery of secondary metabolites (Medema *et al*., 2011c), and **Wayne Mitchell** (Experimental Therapeutics Centre, Singapore) placed synthetic biology in the industrial context, exploring the financial constraints on natural product discovery and the potential of synthetic biology and related technologies to overcome these serious limitations, to provide the necessary secondary-metabolite-based drugs that are required in view of the increased resistance observed in many pathogens worldwide (Mitchell, 2011).

Forward Look Plenary Discussion

At the end of the conference, participants were asked (i) to reflect on the take-home message from the meeting, (ii) to identify challenges and needs of the field, and (iii) and to predict its medium- to long-term future. The spectrum of views was naturally very diverse, but a few important common themes emerged.

Concerning the take home message, there were two recurring views, best reflected in two comments from PhD students: 'Synthetic biology is really just starting' and 'We won't be running out of work any time soon' – there was general enthusiasm about the potential of synthetic biology ('Synthetic biology ideas will continue to *rejuvenate* natural products research in industrial and academic groups'), but also a strong realization that it will be challenging to fully exploit this potential in the rather traditional field of natural products/antibiotics research ('Uptake of synthetic biology approaches by the natural product community is *very* limited at present'). The importance of interdisciplinary conferences bridging the two communities was widely acknowledged, and participants in general expressed their excitement about the learning opportunities offered by bringing together industry, synthetic biologists and natural products researchers.

A number of important needs and challenges in the field were repeatedly identified. They included 'cooperation and communication between biologist, bioinformatics and chemists' (or similar permutations of researchers from various academic and industrial backgrounds like organic and analytical chemists), a 'reduction in the cost of DNA sequencing and assembly' and 'support for interdisciplinary work'. The oral discussion further stressed the need for interdisciplinary training for a new generation of students to facilitate such research across traditional boundaries.

Participants were overall very reluctant to express their view on the future development of the field, reflecting the enormous rapidity of progress that makes viable predictions very hard, even on a 5- to 10-year scale. There was, however, cautious optimism that some major advances will soon be made to overcome the challenges identified above: in 5–10 years 'optimized "general" expression hosts are available', 'there will be synthetic biosynthesis pathways both for natural and (hopefully) unnatural products', 'new natural products will be obtained from heterologously expressed gene clusters' and, perhaps most importantly, 'DNA assembly and synthesis will be cheap and easy'.

It was clear that we are only at the beginning of a new era of biotechnology, which will be driven by a renewed vigour of interdisciplinary interactions. It will be exciting to see how the new ideas and new collaborations forged at this meeting will be turned into practical progress towards

engineering the intricate complexity of antibiotic biosynthesis pathways in the coming months and years.

Acknowledgements

We thank the European Science Foundation, the European Molecular Biology Organization, the Generalitat de Catalunya, the University of Groningen Synthetic Biology Centre and DSM for supporting this meeting. We also thank SGM for sponsoring the speaker prize and *Nature Reviews Microbiology* and *EMBO Reports* for sponsoring the poster prize. Last but not least, we thank all participants who gave permission to include unpublished material in this review and made this meeting such a fruitful and pleasant event.

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